Early and Rapid Diagnosis of Exposure to Biothreat Agents Using Host Gene Expression Responses

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Pathogen specialists / collaborators…..

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  - Luther Lindler (Plague)
  - Robert Putnak (Dengue)
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HOST GENE EXPRESSION RESPONSES TO BIOLOGICAL THREAT AGENTS:

-Rapid and Early Diagnosis of Pathogen Exposure
-Design of Stage-specific Therapeutic Approaches

Traditional approach

- Direct pathogen identification methods are limited due to:

  -dependence on concentration/ time

  -tissue sequestration of pathogen

- Mutants (natural or deliberate) Chimeras, etc, can elude detection by structural-based probes or other direct methods

Host response approach

- Peripheral blood lymphoid cells circulate throughout (minutes):

  -cells encounter pathogens -

  -they respond and create a record of the induced “insults”.

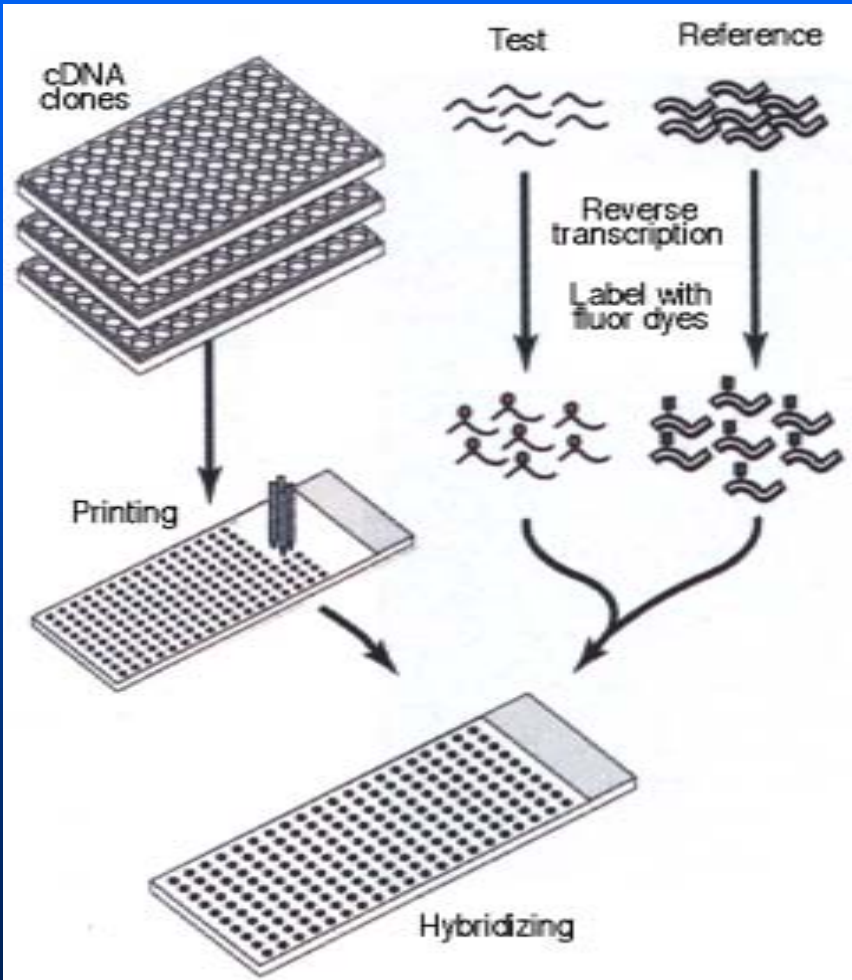
- Draw blood sample, 1-2ml, automatically isolate RNA, gene profile indicates pathogen exposure & stage of illness

Create a library of host gene expression responses to biological threat agents

- using peripheral blood mononuclear cells (PBMC) as the target tissue, isolate RNA, perform microarray analysis on these samples.
- Broadly compare major biological threat agents for multiple exposure time periods *in vitro* to PBMC from healthy donors
- Confirm *in vivo* using monkeys exposed to the threat agent and take PBMC at 3-4 time periods (3 NHP per time period).

TECHNOLOGY INVOLVED:

- Use library of ~40,000 cDNA gene fragments
- Spotted onto 3 slides in duplicate

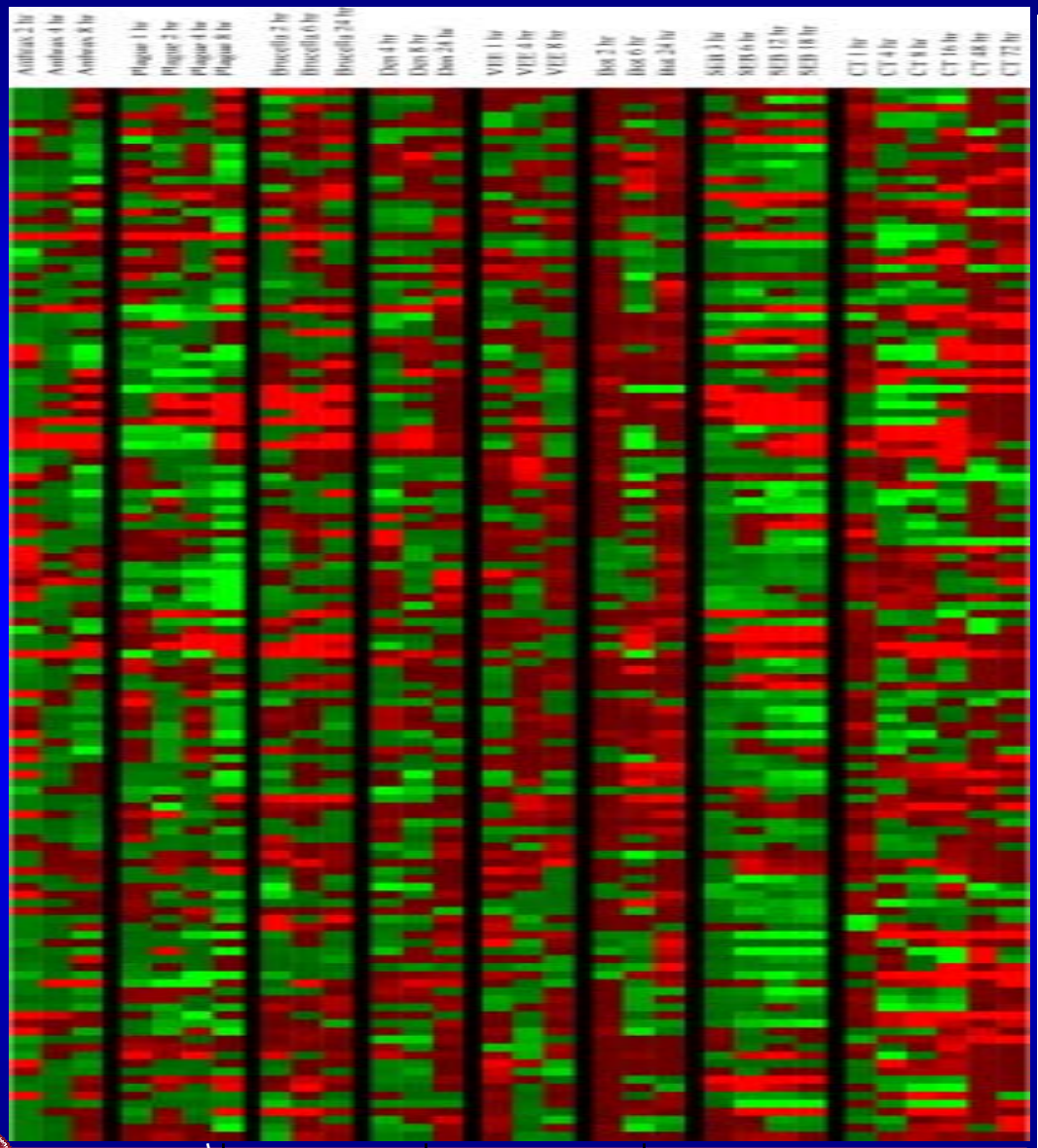


The "test" is compared to a reference sample in order to provide maximum continuity over time.

Control and test are separately compared to the reference.

Reference is a mixture of tumor samples that permits normalization over years of study.

Pathogen induced Gene Profiles



B. anthracis
Y. pestis
Brucella
Dengue
VEE
BOT
SEB
CT

Functional Gene Clusters

ADENYLYL/GUANYULYL CYCLASES & DIESTERASES
APOPTOSIS-ASSOCIATED KINASES
ATPASE TRANSPORTERS
BCL FAMILY
CALCIUM-BINDING PROTEINS
CASPASES
CELL CYLCLE REGULATING KINASES
CELL SURFACE ANTIGENS &ADHESION PROTEINS
CYCLINS
DEATH LIGANDS
DEATH RECEPTOR-ASSOCIATED PROTEINS & ADAPTORS
DNA DAMAGE REPAIR PROTEINS, LIGASES & HELICASES
G PROTEINS
GROWTH FACTOR & CHEMIKI

CYTOKINES

HOMEOSTASIS & DETOXIFICATION
HORMONES
INTERLEUKINS & INTERFERON

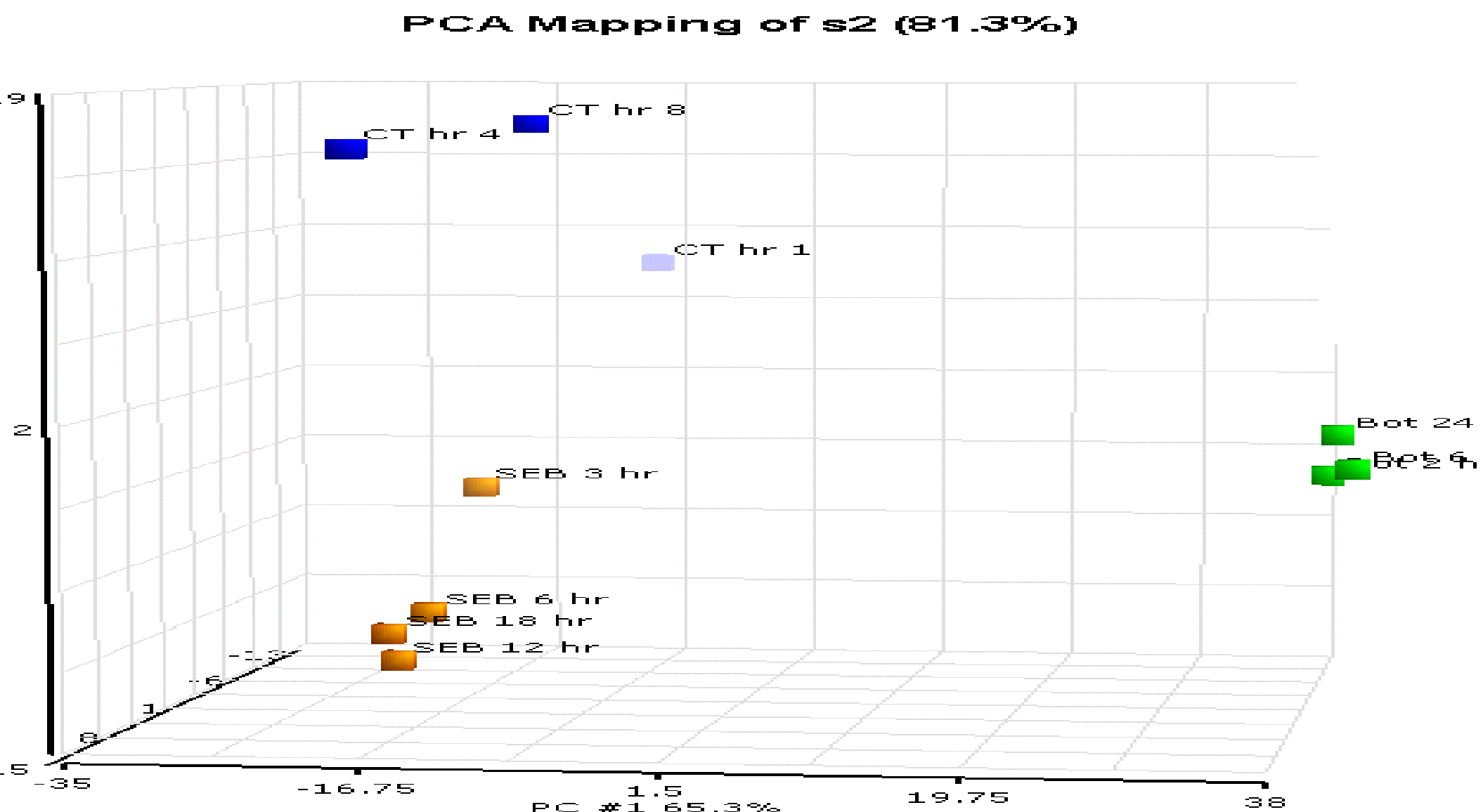
Interleukins

INTERLEUKINS
KINASE SUBSTRATES & INHIBITORS
LIGAND-GATED ION CHANNELS
TRANSCRIPTION FACTORS
NEUROMEDIATORS
NEURONAL DEVELOPMENT & CELL ADHESION
NON-RECEPTOR TYROSINE KINASES
CELL CYCLE REGULATORS
DNA SYNTHESIS, REPAIR & RECOMBINATION PROTEINS
PHOSPHOLIPASES & PHOSPHOINOSITOL KINASES
PROTEIN TURNOVER
SIGNAL TRANSDUCTION RECEPTORS & CELL SURFACE PROTEINS
TRANSCRIPTION FACTOR-RELATED ONCOGENES
TYROSINE KINASE RECEPTORS
TYROSINE PHOSPHATASES

These inflammatory mediators may not differentiate among pathogenic agents, but may still be useful markers to gauge illness progression

Principal Component Analysis

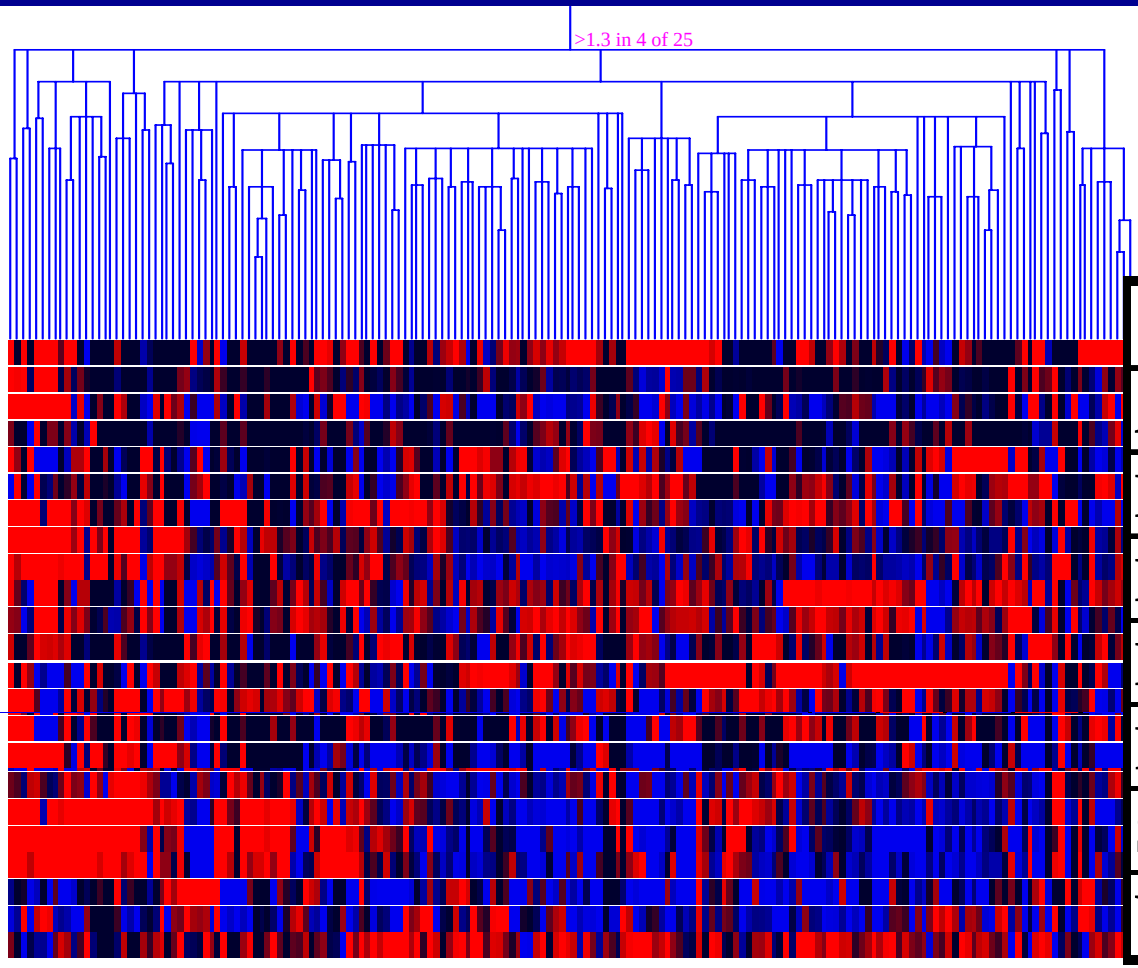
A method for analyzing total gene pattern to illustrate the differences observed for various toxins.



To identify differentiating gene profiles, algorithms were applied

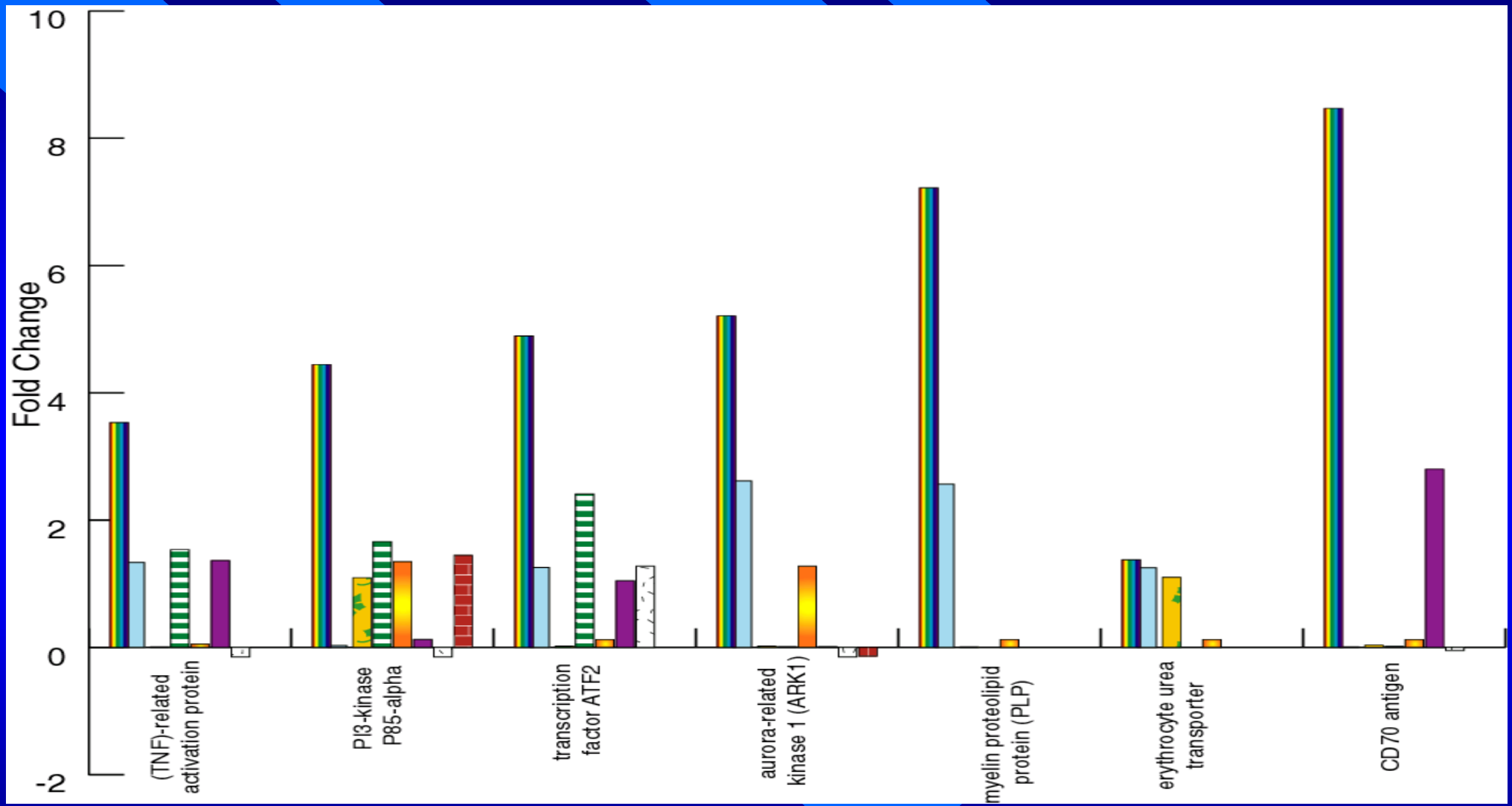
- Statistical power evaluated externally
  - 2-4 experiments for each of 3-5 exposure times per pathogenic agent
- Bonferroni multiple test correction applied to strengthen the reliability of pattern identification

SPECIFIC GENE PATTERNS FOR EACH PATHOGEN



	A	B	C	D	E	F1	F2	G	H	I	J
Anthrax											
BoTox A											
Brucella											
Dengue											
Plague											
SEB											
VEE											

Confirmation of cDNA microarray data



Selection of a few genes that differentiate Brucella exposure from the 8 other pathogens studied

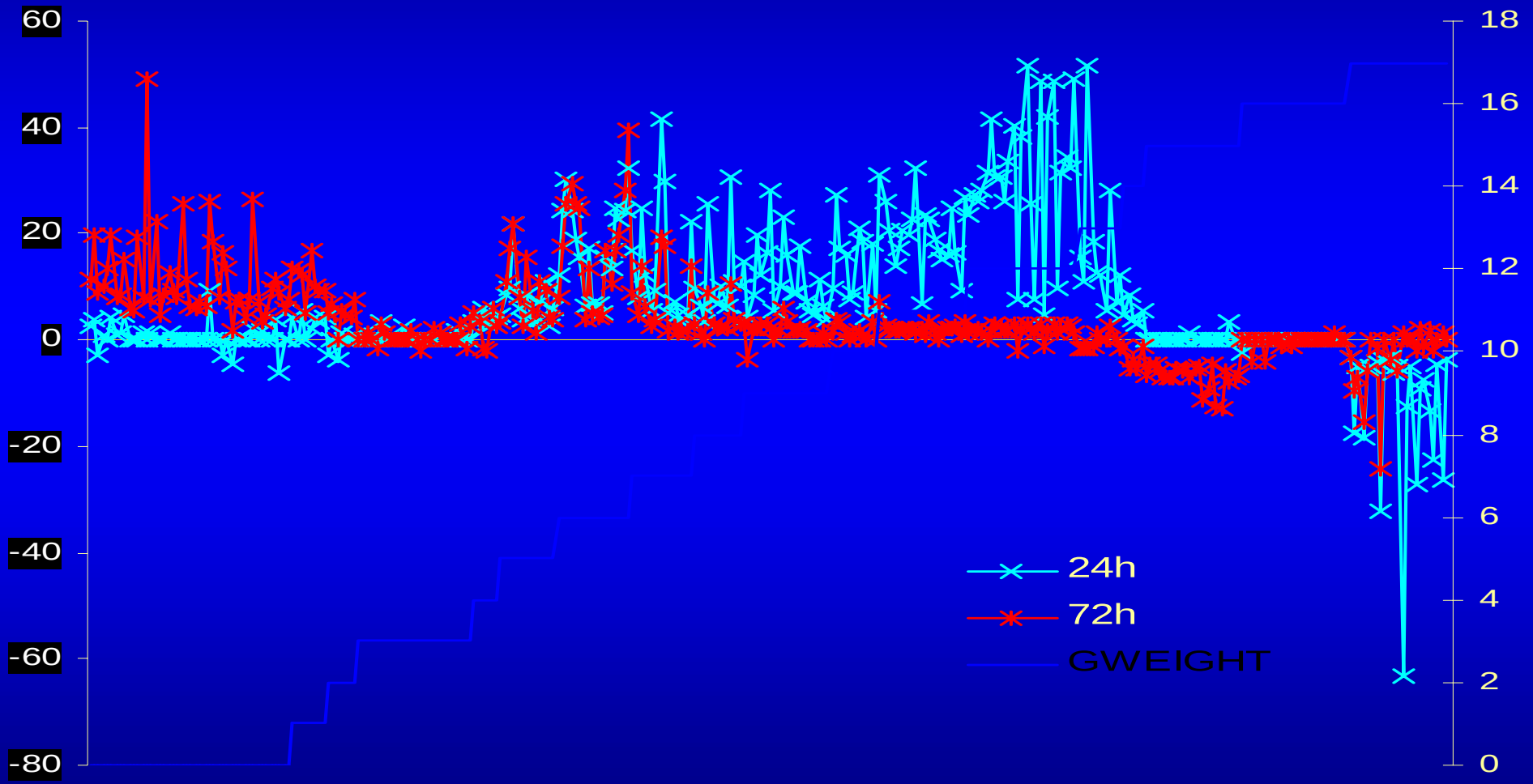
Gene responses as a function of time post-exposure

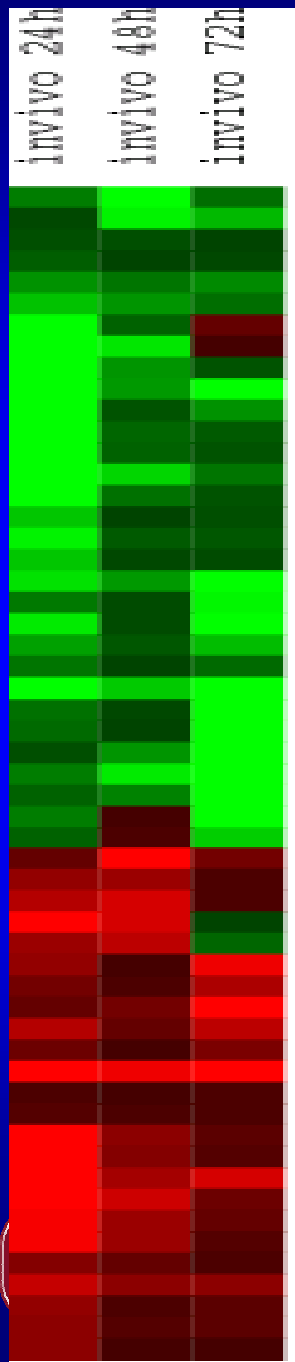
Non-human primate gene patterns

24, 48, 72 h post exposure to B. anthracis at 8 LD_{50}

(mild flu-like illness began ~72 h)

Gene Clustering Analysis in blood samples of NHP exposed to *B anthracis*





OTSG Casualty Projections LTC Deborah Schnelle

Projected survival rates vs time treatment is initiated

Trigger / Decision Points

Medical Surveillance Alert

Clinical Diagnosis

Exposure

PERCENT CASUALTIES AVOIDED (AVERAGE)

100%

80%

60%

40%

20%

0%

100%

71%

29%

12%

Issue Antibiotics Day 0, Exposure
Avoid 100% of Casualties

Day 3, Med Surveillance Alerts
Avoid 71% of Casualties

Day 4, *Avoid 29% of Casualties*

Day 5, Clinical Diagnosis Confirmed
Avoid 12% of Casualties

D0 D1 D2 D3 D4 D5 D6 D7 D9 D12 D14 D18 D21

DAY ANTIBIOTICS INITIATED

Detection by host gene profiling

Clinical diagnosis

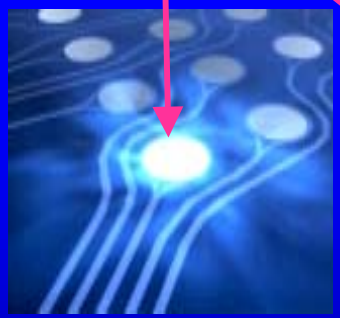
Analysis platform

- The 40,000 gene chip is a splendid research tool but not a viable option in the field
- We envision selecting the “quiet” genes that we have characterized as a basis for diagnosis (~100-1000)
- An electrochemical, microfluidics chip has some of the features we seek-Nanogen
- Metrigenix 4D array platform is another promising technology

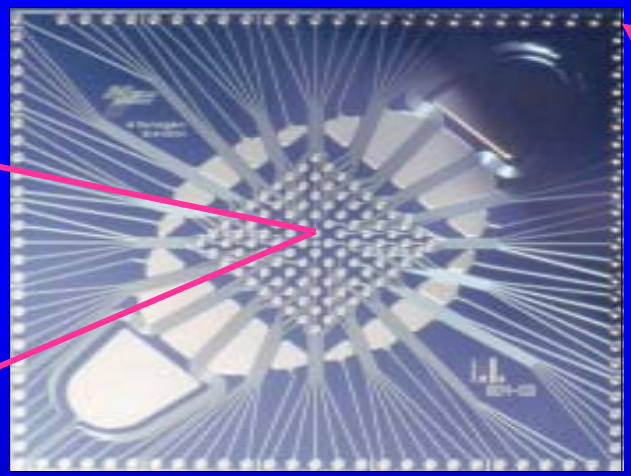
Advantages of Nanogen's Microelectronic Array

- Electronic movement and deposition
- Electronic concentration
- Electronic hybridization

Single test site



Electronic Microarray

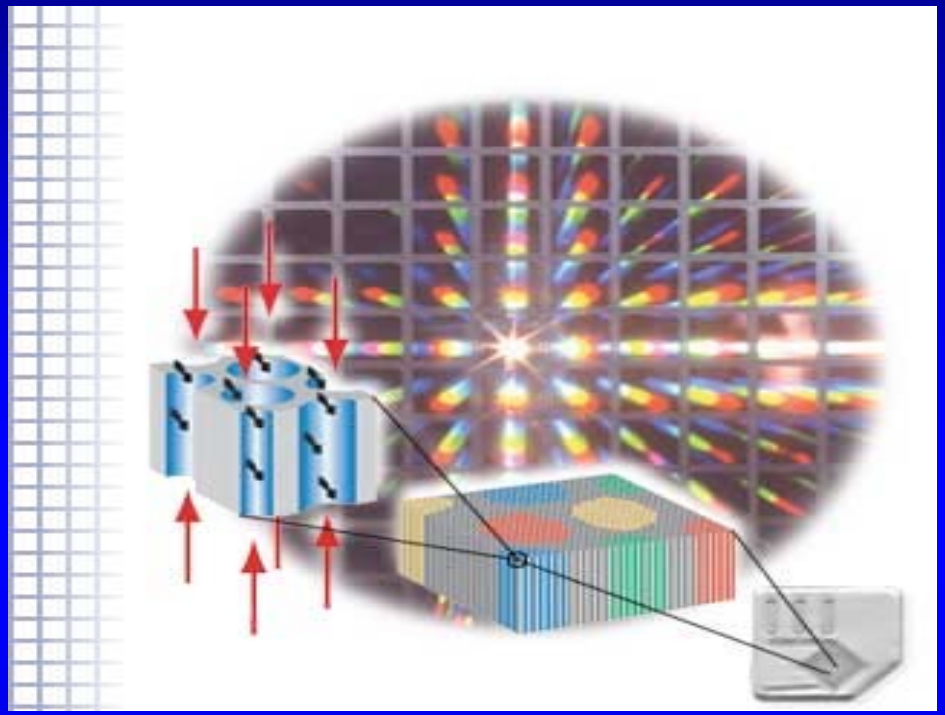


Nanochip Cartridge

(~6 cm long)

The Nanogen gene expression platform could be a very attractive tool in translational research as in tracking biomarkers in clinical samples. It offers the advantage of multiplexing many targets.

Metrigenix Technology



The patented Flow-Thru Chip technology (FTC) serves as the technology base for MetriGenix. The FTC is a state-of-the-art 4-dimensional microarray technology platform that can be used to provide high-throughput, high-content assays to measure gene expression activity. The advantages of developing Flow-Thru Chips include:

- **improved assay responsivity due to the increased surface area**
- **reduced assay times due to enhanced mass-transport within the channels**
- **more uniform spot morphology due to the wetting properties of the microporous chip**
- **smaller sample and reagent volume requirements due to the reduction in the reaction volume**

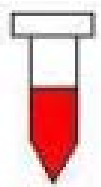
Host Gene Expression Profiles Induced by Biological Threat Agents:

Provides rapid/early diagnosis plus tailored therapeutic approaches

Rapidly compare the gene expression profiles (1000's of genes) with a library of known biological threat and other pathogenic agents

Determine gene pattern to learn: Extent of Individual Exposure, Individual Susceptibility, Course of Impending Illness and Undetectable Potentiating Contaminants

Draw ~2 ml of blood from an exposed individual, use auto-mated system to isolate RNA.



Gene groups

	A	B	C	D	E	F1	F2	G	H	I	J
Anthrax											
BoTox A											
Brucella											
Dengue											
Plague											
SEB											
VEE											

Rapid analysis of exposure. continue monitoring the "negative" profiles

Initiate treatments based on course of impending illness using pattern for agents with similar gene expression profiles.

Standard Dipstick toxin detection method does/does not determine the agent present. Chaos and panic are eminent

Gene Array Technology: Summary

- Uses for Gene Array Technology
  - Attack by an unknown agent
  - Outbreak of an unknown illness
- Specific Diagnosis
  - Causative Agent
  - Degree of Progression of Illness
- Treatment
  - Mechanism of pathologic action of toxin or infection
  - Suggests specific treatments
- The Future
  - Electrochemical detection on microchips
  - Microfluidics and 4D arrays